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(54) Title: ESTERS OF UNSATURATED FATTY ACIDS

(57) Abstract

The isopropyl esters of fatty acids with 16–26 carbon atoms and two to six double bonds in either the cis or the trans configuration.

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ESTERS OF UNSATURATED FATTY ACIDS

Field of the Invention

The invention relates to esters of unsaturated fatty acids.

Glycerides

In the body fatty acids play many important roles as components of membrane structure, as factors in cell signalling systems and as regulators of gene function. The fatty acids are usually taken in the diet in the form of triglycerides, phospholipids and cholesterol esters. The triglycerides are particularly important vehicles for fatty acids. During the process of digestion the fatty acids at the 1 and 3 positions of the triglyceride are split off by pancreatic lipase, leaving a monoacylglycerol with the fatty acid in the 2 position. This monoacylglycerol may be reconverted by acylation to a diglyceride or triglyceride, or the fatty acid may migrate spontaneously to the 1 or 3 positions. Such migration may possibly be enzyme assisted but may also occur non-enzymically since it can occur in vitro in the absence of enzymes.

The 2-monoacylglycerides are important vehicles for the absorption of fatty acids in the small intestine and may be particularly important vehicles for transferring fatty acids into lymphatics which can then take their contents to the blood stream while by-passing the liver. The 2-monoacylglycerides may also have specific biological activities of their own in membranes and in cell signalling systems although as yet these activities are ill-defined.

Present Work

We believe that there is particular value in developing analogues of the 2-monoacylglycerides, which cannot be converted to di or tri-glycerides. Such analogues are effective in delivering the fatty acids to the lymphatic system and therefore into the blood stream without metabolism in the liver, increasing their biological effectiveness.

Glycerol has hydroxyl groups in conventionally numbered 1, 2 and 3 positions to which fatty acids may become attached. If the 2-position is occupied by a fatty acid, that fatty acid may migrate to the 1 or 3 positions, thus changing the properties of the monoester, or other fatty acids may be added to the 1 and 3 positions. 2-propanol is related in structure to glycerol, but has no hydroxyl groups at the 1 and 3 positions. The isopropyl esters of fatty acids are therefore analogous to the monoglycerides with the fatty acid in the two position. However such isopropyl esters cannot be converted to di and tri-glycerides, nor can their structure be changed by internal migration of the fatty acid to the 1 or 3 positions. The isopropyl esters therefore provide novel vehicles for the carriage of fatty acids in biological systems and in particular for their unchanged absorption via the lymphatic system.

The isopropyl esters may be used as delivery systems whereby unsaturated fatty acids may be effectively delivered to the body, particularly by oral and topical but also by other routes. The fatty acids delivered topically in this way are particularly effectively transported across the skin.

Statement of Invention

At its broadest the invention provides the isopropyl esters of fatty acids with 16-26 carbon atoms and two to six double bonds in either the cis or the trans configuration, as such and when for use for example in food, cosmetic and therapeutic applications, all as claimed herein.

Particular examples of the fatty acids include all cis linoleic acid, conjugated linoleic acid, columbinic acid, parinaric acid and all the essential fatty acids of the n-3 and n-6 series, as shown in Table 1.

TABLE 1

n-6 EFAs		n-3 EFAs
18:2n-6 Linoleic acid (LA)		18:3n-3 α-linolenic acid (ALA)
↓	δ-6-desaturase	↓
18:3n-6 γ-Linolenic acid (GLA)		18:4n-3 Stearidonic acid (SA)
↓	elongation	↓
20:3n-6 Dihomo-γ-linolenic acid (DGLA)		20:4n-3 Eicosatetraenoic acid
↓	δ-5-desaturase	↓
20:4n-6 Arachidonic acid (AA)		20: 5n-3 Eicosapentaenoic acid (EPA)
↓	elongation	↓
22:4n-6 Adrenic acid		22:5n-3
↓	δ-4-desaturase	↓
22:5n-6		22-6n-3 Docosahexaenoic acid (DHA)

The acids, which in nature are of the all - cis configuration, are systematically named as derivatives of the corresponding octadecanoic, eicosanoic or docosanoic acids, e.g. z,z octadeca - 9,12 - dienoic acid or z,z,z,z,z,z docosa- 4, 7, 10, 13, 16, 19 - hexaenoic acid, but numerical designations based on the number of carbon atoms, the number of centres of unsaturation and the number of carbon atoms from the end of the chain to where the unsaturation begins, such as, correspondingly, 18:2n-6 or 22:6n-3 are convenient. Initials. e.g., EPA and shortened forms of the name e.g. eicosapentaenoic acid are used as trivial names in some of the cases.

As noted, the esters may be used for a variety of purposes, but particularly as pharmaceuticals, as foods, as nutritional supplements, as food additives and as agents to be

used in all forms of skin and hair care. When administered to humans or animals, they may be given in doses of from 1mg to 100g per day, preferably 10mg to 30g per day and very preferably 100mg to 5g per day. When incorporated into foods, skin care agents or other vehicles they may be used in concentrations from 0.01 to 50% preferably 0.5 to 20%, by weight. They may be formulated in capsules, tablets, emulsions, liquids, creams, ointments, skin patches, pessaries, suppositories or any other vehicle for oral, topical, enteral or parenteral administration known to those skilled in the art.

Isopropyl esters of bioactive fatty acids may be prepared by any reasonable method of ester synthesis and especially:

(a) by reaction of 2-propanol with fatty acid chloride, fatty acid anhydride or suitably activated ester with or without the presence of an organic tertiary base, e.g. pyridine, in a suitable inert solvent, e.g., dichloromethane, at a temperature between -40^{0}C and 120°.

(b) by reaction of fatty acid with an excess of 2-propanol in the presence of a suitable acid catalyst, e.g. concentrated sulphuric acid, with or without an inert cosolvent, e.g. toluene, under reflux conditions.

(c) by reaction of 2-propanol with fatty acid in the presence of a condensing agent, e.g. 1,3-dicyclohexylcarbodiimide, with or without the presence of a suitable organic tertiary base, e.g. 4(N,N-dimethylaminopyridine), in an inert solvent, e.g. dichloromethane, at a temperature between 0° and 50°C.

(d) by reaction of fatty acid or fatty acid activated ester, e.g. vinyl, with an excess of 2-propanol in the presence of a hydrolase enzyme, with or without an inert cosolvent, e.g. hexane, at a temperature between 20°C and reflux.

(e) by reaction of fatty acid with suitable 2-propanol derivative, .e.g. iodide, with or without the presence of a suitable base, e.g. potassium carbonate, in a suitable inert solvent, e.g. dimethylformamide, at a temperature between 0° and 180°C.

The following illustrate particular syntheses of the esters.

EXAMPLE 1

z,z,z-octadeca-6, 9, 12-trienoic acid, isopropyl ester
(ester of GLA and isopropanol)

A solution of 4-(N,N-dimethylamino)pyridine (2.85g, 23.34mmol) and 1,3-dicyclohexylcarbodiimide (4.08g, 19.75mmol) in methylene chloride (15ml) was added to a stirred solution of z,z,z-octadeca-6, 9, 12-trienoic acid (5g, 17.96 mmol) and 2-propanol (1.19g, 19.75 mmol) in methylene chloride (10ml) at room temperature under an atmosphere of nitrogen. On completion of the reaction as shown by tlc, the mixture was filtered. concentrated under reduced pressure and purified by column chromatography to yield z,z,z-octadeca-6, 9, 12-trienoic acid, isopropyl ester as a pale yellow oil.

EXAMPLE 2

z,z,z-octadeca-6, 9, 12-trienoic acid, isopropyl ester
(ester of GLA and isopropanol)

z,z,z-octadeca-6, 9, 12-trienoic acid (5g, 17.96mmol), concentrated sulphuric acid (0.5ml) and 2-propanol (50ml) were heated with stirring under reflux. On completion of the reaction as shown by tlc, the mixture was diluted with hexane (25ml) and neutralised with saturated sodium hydrogen carbonate solution. The organic phase was washed with water (2X12ml). dried with magnesium sulphate, filtered, concentrated under reduced pressure and purified by

column chromatography to yield z,z,z-octadeca-6, 9, 12-trienoic acid, isopropyl ester as a pale yellow oil.

EXAMPLE 3

z,z,z-octadeca-6, 9, 12-trienoic acid, isopropyl ester
(ester of GLA and isopropanol)

z,z,z-octadeca-6, 9, 12-trienoic acid (5g, 17.96mmol), cesium fluoride (5.46g, 35.92 mmol) and 2-iodopropane (6.11g, 35.92 mmol) were heated to 50°C in N,N-dimethylformamide with stirring for 3 days under an atmosphere of nitrogen. The mixture was diluted with ethyl acetate (250ml) and water (100ml). The organic phase was washed with saturated sodium hydrogen carbonate (100ml) and saturated sodium chloride solution (100ml), dried with magnesium sulphate, filtered, concentrated under reduced pressure and purified by column chromatography to yield z,z,z-octadeca-6, 9, 12-trienoic acid, isopropyl ester as a pale yellow oil.

EXAMPLE 4

z,z,z-octadeca-6, 9, 12-trienoic acid, isopropyl ester
(ester of GLA and isopropanol)

z,z,z-octadeca-6, 9, 12-trienoic acid (2g, 7.18mmol), immobilised lipase from Candida antarctica (Novozym-435™ (200mg, 10%w/w) and 2-propanol (40ml) were heated with stirring under reflux. On completion of the reaction as shown by tlc, the mixture was filtered, concentrated under reduced pressure and purified by column chromatography to yield z,z,z-octadeca-6, 9, 12-trienoic acid, isopropyl ester as a pale yellow oil.

EXAMPLE 5

z,z,z,z,z-eicosa-5, 8, 11, 14, 17-pentaenoic acid, isopropyl ester
(ester of EPA and isopropanol)

z,z,z,z,z-eicosa-5, 8, 11,14, 17-pentaenoyl chloride (5g, 15.58mmol) and 2-propanol (50ml) were stirred at room temperature under an atmosphere of nitrogen. On completion of the reaction as shown by tlc, the mixture was concentrated under reduced pressure and purified by column chromatography to yield z,z,z,z,z-eicosa-5, 8, 11, 14, 17-pentaenoic acid, isopropyl ester as a yellow oil.

EXAMPLE 6

z,z,z,z,z-eicosa-5, 8, 11, 14, 17-pentaenoic acid, isopropyl ester
(ester of EPA and isopropanol)

Part 1: cesium z,z,z,z,z-eicosa-5 ,8, 11, 14, 17-pentaenoate
(cesium salt of EPA)

z,z,z,z,z-eicosa-5, 8, 11, 14, 17-pentaenoic acid (5g, 16.53mmol) and cesium carbonate (2.70g, 8.27mmol) were dissolved in methanol (150ml) at room temperature. Methanol was removed under reduced pressure to yield cesium z,z,z,z,z-eicosa-5, 8, 11, 14, 17-pentaenoate as a waxy orange solid.

Part 2: z,z,z,z,z-eicosa-5, 8, 11, 14, 17-pentaenoic acid, isopropyl ester
(ester of EPA and isopropanol)

Cesium z,z,z,z,z-eicosa-5, 8, 11,14, 17-pentaenoate (7.18g 16.53 mmol) and 2-iodopropane (5.62g, 33.06mmol) were heated to 50°C in tetrahydrofuran (55ml) and 1, 3-dimethyl-3, 4, 5, 6-tetrahydro-2(1H)-pyrimidinone (55ml) with stirring for 3 days under an atmosphere of nitrogen. The mixture was diluted with water (50ml) and a 1:1 solution of ethyl acetate and hexane (100ml). The aqueous phase was extracted with ethyl acetate/hexane (1:1, 2X250ml). The combined organic phases were washed with saturated sodium chloride solution (2X50ml) and water (50ml), dried with magnesium sulphate, filtered, concentrated under reduced pressure and purified by column chromatography to yield z,z,z,z,z-eicosa-5, 8, 11, 14, 17-pentaenoic acid, isopropyl ester as a yellow oil.

EXAMPLE 7

Using the above methods, isopropyl esters of the n-6 EFAs other than GLA, of the n-3 EFAs other than EPA, and of conjugated linoleic acid, parinaric acid and columbinic acid are prepared.

The following are formulation examples for administration for the purposes and in the dosages set out herein.

EXAMPLE 8

Capsules, hard or soft gelatin, containing 400mg of the isopropyl ester of linoleic acid, alphalinolenic acid, gamma-linolenic acid, arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid, or any of the other fatty acids specified.

EXAMPLE 9

Emulsions for oral or intravenous administration containing 10% by weight of the isopropyl ester of a fatty acid as mentioned in Example 8, suitable natural, synthetic and semi-synthetic emulsifiers, such as phospholipids and galactolipids, known to those skilled in the field being used. Galactolipid emulsifiers may in particular be those of our PCT patent application SE 97/00115 (WO 95/20943)

EXAMPLE 10

Creams or ointments for topical application prepared as per se known to those skilled in the field and containing 1% by weight of the isopropyl ester of a fatty acid as mentioned in Example 8.

CLAIMS

1. The isopropyl esters of fatty acids with 16-26 carbon atoms and two to six double bonds in either the cis or the trans configuration.

2. An ester of an n-6 or n-3 essential fatty acid, conjugated linoleic acid, columbinic acid, or parinaric acid, as in claim 1.

3. A fatty acid ester as in claim 1 or 2, as an acceptable preparation suited to administration of 1mg to 100g preferably 10mg to 30g, very preferably 100mg to 5g of the fatty acid daily.

4. A fatty acid ester as in claim 1 or 2, as an acceptable preparation for topical application, or as a food, food additive or nutritional supplement, the preparation comprising the fatty acid in the concentration of 0.01 to 50% by weight.

5. When for use in therapy, a fatty acid ester as in claim 1 or 2.

6. When for use in a cosmetic, skin or hair care preparation or in a food, food additive or nutritional supplement, a fatty acid ester as in claim 1 or 2.

7. A method of preparation of a composition for application to the skin in a cosmetic, skin or hair care context or in treatment of dermatological disorders or in delivery of fatty acids to the bloodstream avoiding the hepatic circulation, wherein use is made of an ester as in claim 1 or 2.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 97/02502

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07C69/587 A61K7/06 A61K31/23 A23D7/00

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 00107 A (AMINCO INC.) 5 January 1995 see page 3, line 25 - page 4, line 5 see page 5, line 11 see page 20 - page 23; claims ---	1,2,6,7
X	DATABASE WPI Week 9351 19 November 1993 Derwent Publications Ltd., London, GB; AN 93-408837 XP002047562 & JP 05 306 223 A (MAEDA YAKUHIN KOGYO KK) , 19 November 1993 see abstract --- -/--	1,2,6,7

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Name and mailing address of the ISA	Authorized officer
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International Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 59, no. 1, 8 July 1963 Columbus, Ohio, US; abstract no. 433e, STEFAN KOVAC: "The preparation of alkyl esters of cis,cis-delta 9,12-octadecadienoic acid and cis,cis,cis-delta 9,12,15-octadecatrienoic acid." page 263; column 433; XP002047559 see abstract & CHEM.ZVESTI, vol. 16, 1962, pages 82-88, ---	1,2
X	CHEMICAL ABSTRACTS, vol. 123, no. 5, 31 July 1995 Columbus, Ohio, US; abstract no. 47841y, SAKAI HIROYUKI ET AL.: "Effect of topical docosahexaenoic acid (DHA) isopropyl ester on retinal function in vitamin E-deficient rabbits" page 79; column left; XP002047560 see abstract & ATARASHII GANKA, vol. 12, no. 4, 1995, pages 683-688, ---	1,2
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Information on patent family members

International Application No
PCT/GB 97/02502

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